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Folding transition into a loosely collapsed state in plasmid DNA as revealed by single-molecule observation

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Abstract The conformational transition of a plasmid DNA, pGEG.GL3 (12.5 kbp, circular), induced by spermine(4+) was studied through the observation of individual DNA by fluorescence microscopy. We deduced the change in the hydrodynamic radius R_H from an analysis of the Brownian motion of single DNA molecules. R_H decreases in a continuous manner with an increase in spermine(4+), in contrast to the large discrete on/off change for long linear DNA. Just after the transition to the collapsed state, a small number of DNA molecules tend to form an assembly, which disperses in the bulk solution without precipitation.

Keywords: Circular DNA; DNA condensation; Single DNA observation; Colloidal particles; DNA assembly; Spermine; DNA morphology

1. Introduction

In living cells and viruses, giant DNA molecules are folded into a tightly packed compact state. It is natural to expect that the biological activity of these giant DNA molecules should be highly dependent on their higher-order structure [1]. Not a small amount of studies on the folding transition of giant DNA molecules into condensed states, i.e., "DNA condensation", have been reported [2,3]. According to the standard understanding in polymer science concerning the conformation of a polymer chain [4], the compact state is generated under poor solvent conditions. Thus, it has been thought that the phenomenon of compaction in a single polymer chain be realized only under very dilute conditions, and that aggregation between many polymer chains is unavoidable in actual experimental conditions. Under such circumstances, DNA condensation has been regarded as a mixed process with characteristics of both single-chain compaction and multiple-chain aggregation [2]. About a decade ago, it was clarified that, from experiments on the observation of single DNA molecules, large linear dsDNA molecules larger than on the order of several tens of kbp undergo a large discrete transition between an elongated coil state and a folded compact state upon the addition of various kinds of condensing agents, such as polyamines, multivalent metal cations, hydrophilic polymer, and cationic surfactants [5,6]. As an important property of compact DNA, it has been shown that individual compact DNA molecules behave as colloidal particles with a negative charge and do not adhere to each other even after they collide [7,8]. Thus, a higher concentration of condensing agents is necessary to cause the aggregation/precipitation of multiple DNA molecules than is needed for the compaction of single DNA.

In contrast to such a significant change in linear giant DNA, it is still unclear how circular DNA folds into a compact state. A few reports on the conformation of circular DNA based on AFM observations have recently been published [9,10]. Most of the episome and genomic DNA in prokaryotes has a circular structure. In addition, even for eukaryotes, the transcriptionally active part of giant DNA is considered to be unfolded from its scaffold, suggesting the formation of a circular structure [11,12]. The present study was performed to better understand the transition in the higher-order structure of circular DNA molecules.

2. Materials and methods

2.1. Materials

Plasmid DNA pGEG.GL3 (12.5 kbp; circular DNA) was prepared as described previously [13]. 4′,6-Diamino-2-phenylindole (DAPI) was purchased from Wako Pure Chemicals. Dithiothreitol (DTT) and spermine tetrahydrochloride (SPM) were obtained from Nakalai Tesque.

2.2. Fluorescence microscopic measurements

Plasmid DNA pGEG.GL3 was dissolved in TE buffer solution (pH 8.0, 10 mM Tris–HCl, and 1 mM EDTA). The final concentrations in the sample for fluorescence microscopic observation were as follows: 0.18 μM pGEG.GL3, 0.18 μM DAPI and 5 mM DTT. Fluorescent images of DNA molecules were observed with a Zeiss Axiovert 135 TV microscope and recorded through a Hamamatsu Photonics EBCCD camera and an Argus 10 image processor.

2.3. Laser trapping

The infrared laser used for optical trapping was a Nd: yttrium–aluminum garnet, YAG, laser (SL902T, Spectron), with a TEM_{00} beam at a wavelength of 1064 nm. The laser beam was reflected by a dichroic mirror, and focused through an objective lens (Nikon Plan Fluor;

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100×, NA = 1.30) with a microscope (Nikon TE-300). The beam power was 510 mW, as evaluated just before the objective lens with a laser power meter (Neoark PM-345).

3. Results and discussion

Fig. 1A shows fluorescent micrographs of the plasmid DNA pGEG.GL3; (a) without SPM, (b) with 10 μM SPM, and (c) with 13 μM SPM, where the middle column shows the tracks of Brownian motion of the objects for up to 0.3 s. The pictures on the right are quasi-three-dimensional representations of the spatial distribution of the fluorescent intensity. DNA molecules clearly show a dramatic change upon the addition of SPM; in the absence of SPM, the fluorescence image is rather shallow and Brownian motion is mild, whereas in the presence of SPM a bright spot with large thermal fluctuation appears. From such a marked change in fluorescence, we can easily distinguish the compact state of a single DNA from an assembly of multiple DNA molecules. With an increase in the SPM concentration, the DNA molecules tend to assemble/aggregate with each other, as exemplified in Fig. 1A(c).

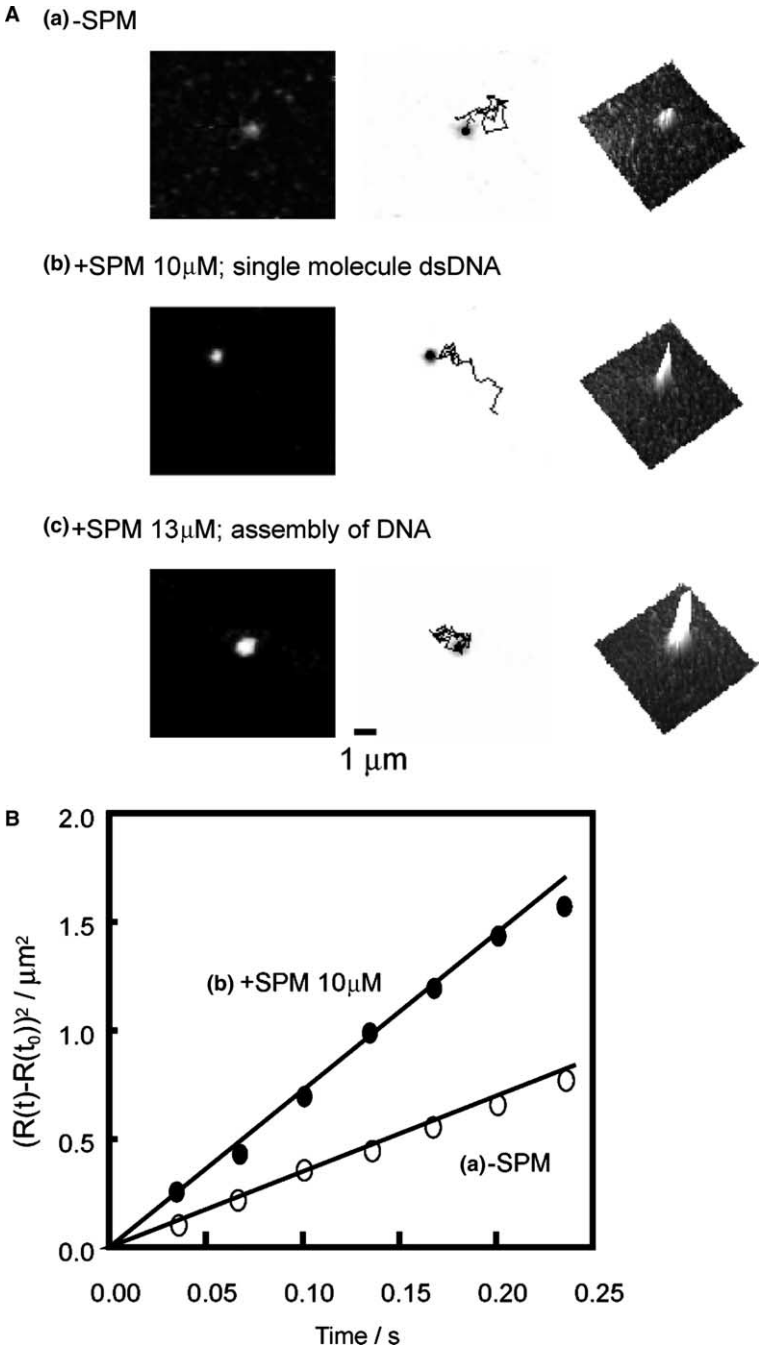

Fig. 1. (A) Differences in the elongated coil state, folded compact state and assembly of multiple DNAs. Left; fluorescent microscopic images of circular plasmid DNA, pGEG.GL3. Middle; tracks of fluorescent objects for 0.3 s. Right; quasi-three-dimensional representation of fluorescent intensity. (B) Mean square displacement of plasmid DNA without and with SPM (10 μM).

Fig. 1B shows a plot of the mean square displacement of DNA vs. time (a) with 10 μM SPM and (b) without SPM. The diffusion constant D for an individual fluorescent object can be deduced from the slope of the linear relationship using the following relationship [14]:

$$\langle(\mathbf{R}(t) - \mathbf{R}(t_0))^2\rangle = 4D(t - t_0), \quad (1)$$

where $\mathbf{R} = (R_x, R_y)$ is a two-dimensional vector indicating the spatial position of the DNA particle. The hydrodynamic radius R_H is calculated from D based on the Stokes–Einstein relation:

$$R_H = k_B T/6\pi\eta_s D, \quad (2)$$

where k_B is the Boltzmann constant and η_s is the viscosity of the solvent. With this treatment of the experimental data, it is possible to evaluate the spatial extension, or degree of swelling/compaction, of individual DNA molecules in a quantitative manner.

Fig. 2 summarizes the results of the analysis of Brownian motion, where histograms of the hydrodynamic radius R_H of pGEG.GL3 at various SPM concentrations are shown; 36 DNA molecules were counted at each concentration. The distribution profile is unimodal for a SPM concentration up to 10 μM, indicating that the transition from the elongated coil to the compact state is mild and continuous. This continuous nature of the transition is markedly different from the transition of linear giant DNA, where a bimodal size distribution is observed in the transition region [6]. The size distribution at 13 μM SPM is markedly different for those at lower SPM concentrations. Based on a careful inspection of the fluorescent intensity and the degree of Brownian motion (see Fig. 1), it seems that the broad peak at the larger R_H values can be attributed to the assembly or clustering of multiple DNA molecules.

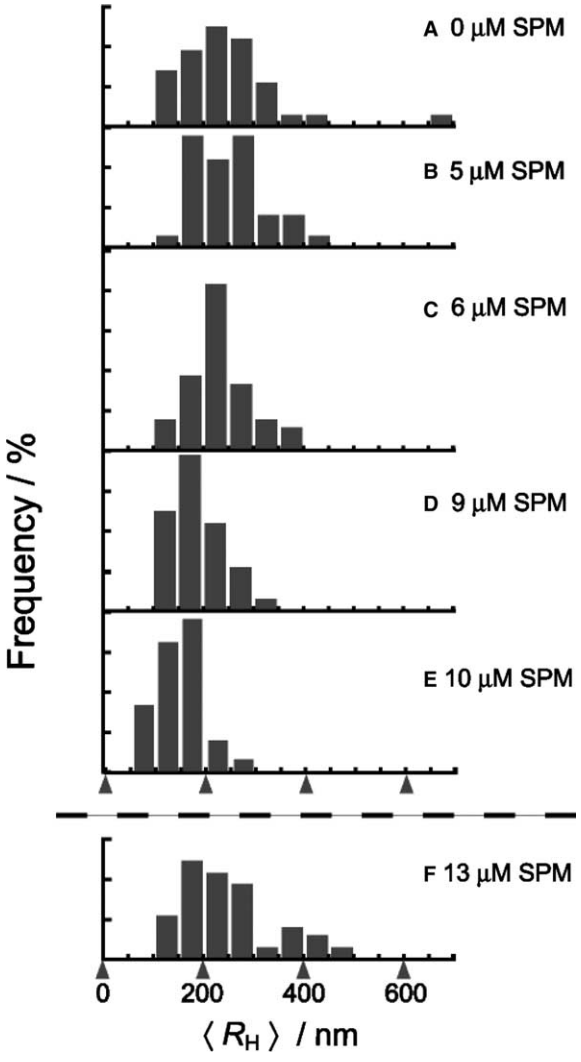

Fig. 2. Distribution of the hydrodynamic radius, R_H, of plasmid DNA at various SPM concentrations, deduced from the analysis of Brownian motion on single DNA molecules. The region denoted by the broken line corresponds to the generation of assemblies with multiple collapsed DNA molecules.

Fig. 3 shows the change in the average value of R_H depending on SPM below 10 μM, where a single DNA molecule is dispersed without aggregation. R_H changes about 40% between the elongated (260 nm) and compact states (150 nm): the difference in density is on the order of $1.4^3 \approx 3$. This is in marked contrast to the large discrete transition in the case of linear giant DNA, where the difference in density is on the order of 10^3–10^4 [15,16]. This small difference in density in the present study indicates that the collapsed plasmid DNA is rather loosely condensed. On the other hand, the collapsed product of giant linear DNA is characterized as a tightly packed state with an ordered morphology, such as a toroid or rod [17,18]. For example, the R_H values of the coil and compact states of T4 DNA, a linear giant DNA of 165 kbp, have been shown to be 900 and 90 nm [16].

Next, we examined the stability of multiple-chain assemblies observed at an SPM concentration above 10 μM. By using laser trapping as an experimental tool, a DNA assembly can be

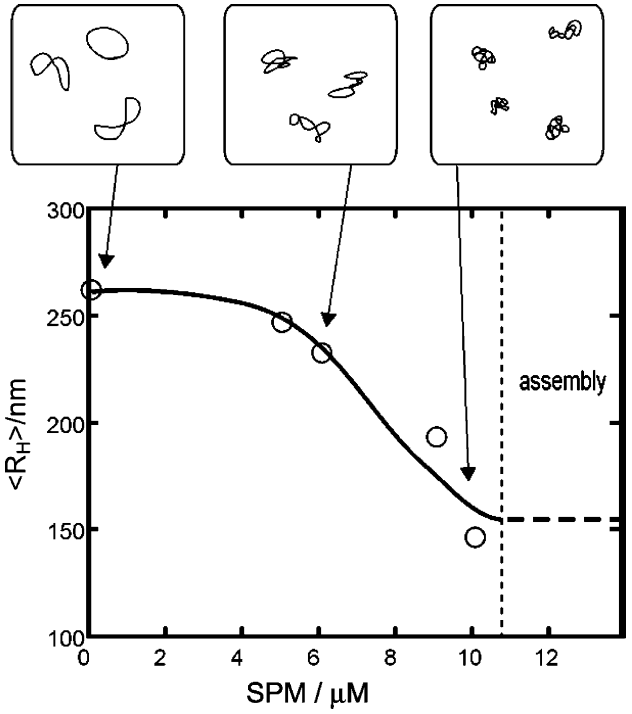

Fig. 3. Change in the average hydrodynamic radius, $\langle R_H \rangle$, of plasmid DNA with a schematic representation as a function of the concentration of SPM. $\langle R_H \rangle$ is the ensemble average for 36 DNA molecules at each data point.

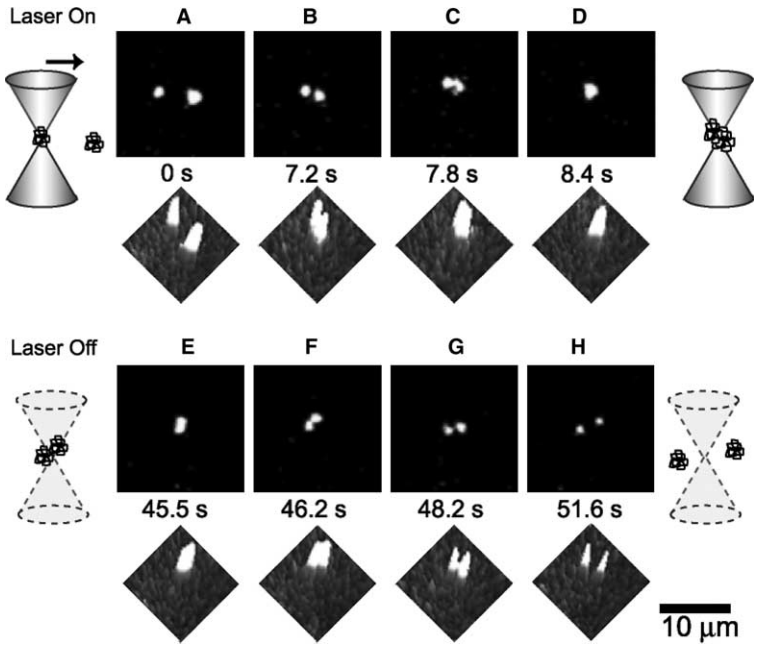

Fig. 4. Experimental results with laser trapping of the assembly of plasmid DNA molecules at 11 μM SPM. (A)–(D) A pair of DNA assemblies are forced in contact with each other by the laser tweezers. (E)–(H) Just after the laser is switched off, the assemblies dissociate and disperse into the bulk solution.

fixed at the focus of the IR laser [8]. As shown in Fig. 4, we collected a pair of assemblies at the focus and annealed for more than 30 s. The laser was then switched off to release the trapped assemblies into the bulk solution. Fig. 4E–H clearly shows that the assemblies trapped at the focus are released as individual particles to disperse into the bulk. This experimental observation reveals the colloidal nature of the assemblies, i.e., individual assemblies are thermodynamically dispersed due to the negative charge in the compact state. If we consider the remaining charge on individual DNA molecules as q, the free energy of an assembly composed of n DNA molecules (related to the radius of assembly $r \sim n^{1/3}$) can be expressed simply as [19]

$$F = -ar^3 + br^2 + c\frac{(nq)^2}{r} \sim -an + bn^{2/3} + cn^{5/3}, \quad (3)$$

where a, b, and c are positive constants. The first, second and third terms correspond to the volume energy, surface energy and Coloumbic energy, respectively. The above equation indicates the existence of a free energy minimum at a certain n value, and the number of DNA molecules in an assembly decreases with an increase in the surviving negative charge, or with loosening of the compact state. It may be useful at this point to note a recent observation that charged colloids can form a stable assembly with a low aggregation number in solution [20].

It has been reported that the efficiency of gene expression for circular DNA (4 kbp) increased with an increase in the concentration of polycation and polyarginine [21]. On the other hand, it has also been shown that the transcriptional activities of λZAP II DNA [22] and chloroplast DNA [23] were completely suppressed with compaction in an in vitro experiment. It may be possible to explain these opposite effects of condensing agents on genetic activity by considering the difference in the nature of packing; when the packing is loose/tight, the activity of transcription and also expression becomes promoted/inhibited, respectively. Notably, circular DNA in living organisms generally shows supercoiling [24], which may be important to make clear the properties of DNA as a macromolecule [25,26].

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